Culture media and IVF/ICSI success rates

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History of culture media

- First embryo cultures by Edwards
  - physiological salt solutions
  - Cell culture, mouse culture
  - Earle's, Ham's F10, T6, WM1
- 1984-1985: media specifically for human IVF
  - Human tubal fluid analysis
  - MB2, HTF
- Aminoacids, vitamins, chelators, antibiotics, growth factors

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Many culture media

Company	Culture media
Irvine Scientific	HTF, P1, MultiBlast, CSCM, ECM
Vitrolife	G2, G3, G5, CCM
MediCult (Origio)	Universal IVF, BlastAssist, ISM, EmbryoAssist, EmbryoGen
Cook Medical	Sydney IVF cleavage/blastocyst medium
Sage	Quinn's advantage cleavage/blastocyst medium
Scandinavian IVF	IVF (Vitrolife)
InVitroCare	HTF, IVC1-3
IVF Online	Global
Ellios Bio-Media	EllioStep2, BM1, SMART2
Api-System	Menezo B2
Gynemed	GM501

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Which medium is the best?

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Current way of choosing...

- UMCN
  - HTF
  - Good results and it is cheap. Other media tried in the past with similar results. In January try Sage
- UMCG
  - G1/G2 (from 2009), in the past HTF
  - PGD (culture day 4/5) and experience from Maastricht
- VUmc
  - Sage Quinn's advantage (2009)
  - Metabolomics study showed HTF was not constant in constituents within same batches. Started looking for sequential media and ended up with 2 candidates: Vitrolife and Sage. After asking around in Belgium and the US and because Sage was cheaper than Vitrolife, Sage was chosen with very good results.

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Current way of choosing...

- MUMC
  - Vitrolife media (since 1995/1998?)
  - At that time, one of the very few commercially available media. At the moment also using Sage, as a prelude to the medium study part 2.
- Catharina ziekenhuis
  - Vitrolife (December 2010)
  - Not good results from HTF, Medium study
- AMC
  - Switched from Ham's F10 to HTF (1999), then Vitrolife (2010), now SAGE (2013)
  - Wished to no longer make the medium but buy it commercially. HTF was chosen as one of the few media available and it was tested in Nijmegen so it felt good. Vitrolife was chosen based on data from Maastricht. SAGE based on good results in the VU.

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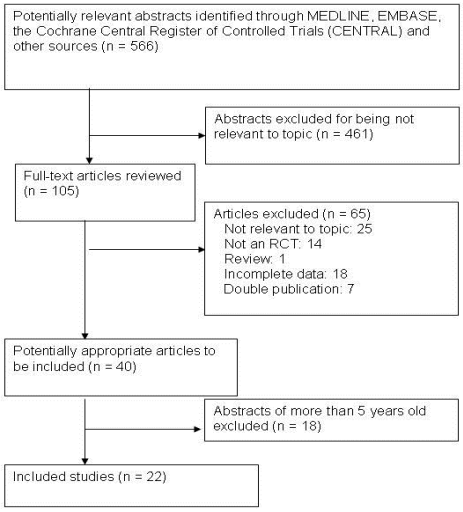
Systematic review and meta analysis

- Aim of the review
  - What medium is best?
    - Live birth / ongoing pregnancy
  - Help the embryologist make informed choices
- Inclusion criteria
  - randomized controlled trials comparing different embryo culture media
    - Randomising women
    - Randomising oocytes/embryos

Methods/Protocol

- Primary outcome
  - Live-birth rate (LBR)
- Secondary outcomes
  - Ongoing pregnancy rate (OPR) per randomised woman
  - Clinical pregnancy rate per randomised woman
  - Miscarriage rate per randomised woman
  - Multiple pregnancy rate per woman randomised
  - Implantation rate per embryo transferred
  - Cryopreservation rate per randomised woman
  - Number of top quality embryos
  - Fertilization rate per oocyte retrieved
  - Health of babies born

Systematic review of the literature



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Quality of studies

Table II: Quality assessment of the studies included.

	Quinn 1985	Parinaud 1998	Mauri 2001	Utsunomiya 2002	Zollner 2004	Ben-Josef 2004	Summers-Chase 2004	Balaban 2005	Hoogendijk 2007	Sepulveda 2009	Campo 2010	Dumoulin 2010	Paternot 2010	Staessen 1998	Khoury 2012	Nelissen 2012	Parinaud 1999	Artini 2004	Findikli 2004	Reed 2009	Hambiliki 2010	Di Falco Cossiello 2011
	Studies randomizing women/cycles																Studies randomizing oocytes/embryos					
Full paper																						
Allocation concealment																						
Blinding																						
Randomization																						
Power calculation																						
ITT																						
Overall study quality	-	+/-	+	-	+/-	+/-	+/-	+/-	+/-	-	+	+	+	+/-	+	+	-	+/-	-	-	+/-	+

= yes, = no, = unknown, + = good, +/- = moderate, - = poor

ITT = intention to treat protocol

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Reported outcomes

Included studies	Compared media	Pregnancy outcomes						Embryo outcomes			
Studies randomizing women		**LBR**	**HN**	**OPR**	**CPR**	**MR**	**MPR**	**FR**	**EQ**	**CR**	**IR**
Quinn 1985	HTF vs. T6†				x						
Parinaud 1998	EllioStep2† vs. BM1† vs. IVF				x				x[1]		x
Mauri 2001	P1 vs. IVF					x		x	x[1]		x
Utsunomiya 2002	HTF/MultiBlast vs. G2† vs. HTF/Sydney IVF			x	x	x	x	x			x
Zollner 2004	G2† vs. BlastAssist	x			x	x	x	x	x[1]		x
Ben-Josef 2004	P1 vs. Sydney IVF	x[1]			x[1]		x[1]	x[1]	x		x
Summers-Chase 2004	HTF vs. P1 vs. Quinn's				x[1]		x[1]				x
Balaban 2005	G3† vs. G2†				x		x	x	x		x
Hoogendijk 2007	Sydney IVF vs. Quinn's			x[1]				x[1]	x		
Sepulveda 2009	Global vs. MultiBlast			x	x	x			x[1]		x
Campo 2010	ISM1 vs. GM501	x		x	x	x		x		x	x
Dumoulin 2010	Sydney IVF vs. G3†	x[2]	x[2]		x[2]	x[2]	x[2]	x[1]	x[1]		x
Paternot 2010	Sydney IVF vs. GM501	x		x	x	x	x	x	x	x[1]	x
Khoury 2012	Global vs. Quinn's				x[1]			x		x	x
Nelissen 2012	Sydney IVF vs. G3†	x	x		x	x	x				
Studies randomizing oocytes/embryos											
Staessen 1998	MB2† vs. Universal IVF vs. BM1†				x[3]	x[3]	x[3]	x			x
Parinaud 1999	EllioStep2† vs. SMART2†							x	x		
Artini 2004	HTF vs. P1				x[1,3]			x	x		x
Findikli 2004	ISM vs. G2†							x	x[1]		
Reed 2009	Global vs. G5			x[1,3]	x[1,3]			x[4]	x[1]		x[1]
Hambiliki 2010	EmbryoAssist vs G5	x[1]			x[1]			x[4]	x	x	x
Di Falco Cossiello 2011	HTF vs. Universal IVF vs. Global vs. IVF								x		

[1]the exact number of women/oocytes/embryos were not known, [2]double publication, [3]oocyte/embryo randomization, [4]fertilization was performed in other media

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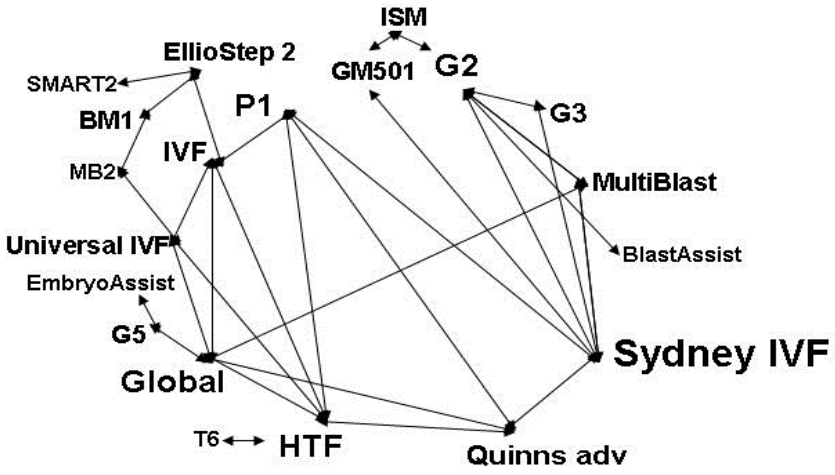
Comparisons made



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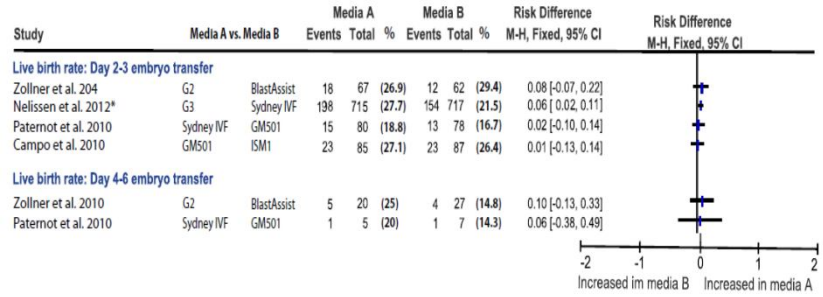
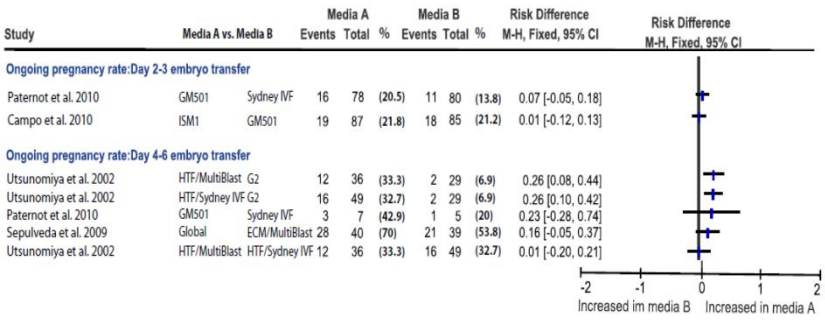
Live birth rate



Mantikou et al, Hum Reprod Update, in press

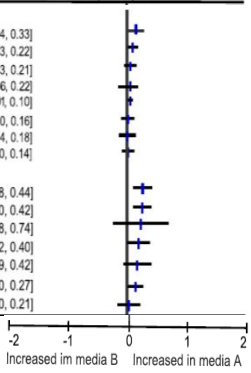
Ongoing pregnancy rate



Mantikou et al, Hum Reprod Update, in press

Clinical pregnancy rate

Study	Media A vs. Media B		Media A Events	Total	%	Media B Events	Total	%	Risk Difference M-H, Fixed, 95% CI
Clinical pregnancy rate: Day 2-3 embryo transfer									
Quinn et al. 1985	HTF	T6	18	60	(30)	6	53	(11.3)	0.19 [0.04, 0.33]
Balaban et al. 2005*	G3	G2	100	199	(50.3)	76	201	(37.8)	0.12 [0.03, 0.22]
Paternot et al. 2010	GM501	Sydney IVF	18	78	(23.1)	11	80	(13.8)	0.09 [-0.03, 0.21]
Parinaud et al. 1998	EllioStep 2	IVF	50	100	(50)	42	100	(42)	0.08 [-0.16, 0.22]
Nelissen et al. 2012*	G3	Sydney IVF	210	715	(29.4)	168	717	(23.4)	0.06 [0.01, 0.10]
Campo et al. 2010	ISM1	GM501	22	87	(25.3)	19	85	(22.4)	0.03 [-0.10, 0.16]
Zollner et al. 2004	BlastAssist	G2	20	62	(32.3)	20	67	(29.9)	0.02 [-0.14, 0.18]
Parinaud et al. 1998	BM1	EllioStep 2	32	108	(29.6)	30	108	(27.8)	0.02 [-0.10, 0.14]
Clinical pregnancy rate: Day 4-6 embryo transfer									
Utsunomiya et al. 2002	HTF/MultiBlast	G2	12	36	(33.3)	2	29	(6.9)	0.26 [0.08, 0.44]
Utsunomiya et al. 2002	HTF/Sydney IVF	G2	16	49	(32.7)	2	29	(6.9)	0.26 [0.10, 0.42]
Paternot et al. 2010	GM501	Sydney IVF	3	7	(42.8)	1	5	(20)	0.23 [-0.28, 0.74]
Sepulveda et al. 2009	Global	ECM/MultiBlast	29	40	(72.5)	21	39	(53.8)	0.19 [-0.02, 0.40]
Zollner et al. 2004	G2	BlastAssist	7	20	(35)	5	27	(18.5)	0.16 [-0.09, 0.42]
Balaban et al. 2005*	G3	G2	48	100	(48)	34	99	(34.3)	0.14 [0.00, 0.27]
Utsunomiya et al. 2002	HTF/MultiBlast	Sydney IVF	12	36	(33.3)	16	49	(32.7)	0.01 [-0.20, 0.21]



Mantikou et al, Hum Reprod Update, in press

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Unconventional Meta-analysis

Medium A			Medium B			Risk Difference			
medium	Events	Total	# of media compared with	Events	Total	RD	CI	I^2 (%)	Risk Difference M-H, Random, 95% CI
G2	141	445	4	201	473	-0.12	[-0.22, -0.03]	57	
Sydney IVF	212	900	4	245	865	0.01	[-0.12, 0.14]	80	
MultiBlast	45	111	3	47	118	0.03	[-0.23, 0.30]	82	
EllioStep2	80	208	2	74	208	0.02	[-0.07, 0.12]	11	
GM501	40	170	2	34	172	0.04	[-0.06,0.14]	18	

Mantikou et al, Hum Reprod Update, in press

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Discussion

- No proper meta-analysis was possible
  - Many media/comparisons, poor quality of studies/reporting
- Guidelines for further research
  - Very few studies report ong. pregnancy / life birth
  - Methodological limitations
    - Randomization protocol
    - Randomization of oocytes/embryos
    - Small sample size
  - Outcome reporting limitations
    - Percentages/ means
    - Heterogeneity in definitions used
- Proper evidence-based introduction of new media
  - In YOUR laboratory
  - By industry

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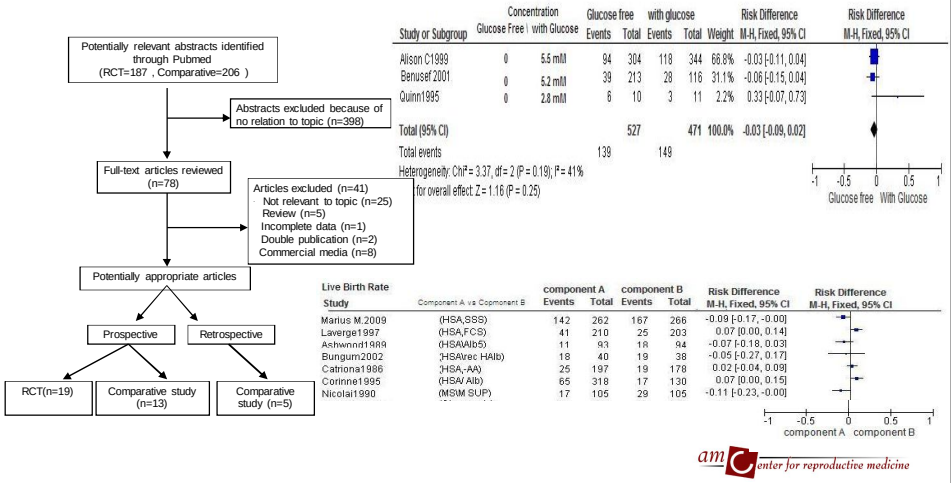
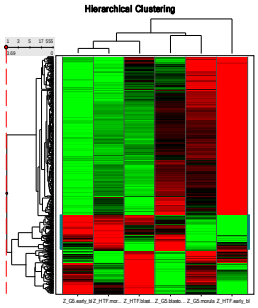
Relevant ongoing research

- Review on components of culture media



Relevant ongoing research

- Review on components of culture media
- Medium study 1
  (AMC/MUMC/UMCG/UMCN/Catharina/Elizabeth)
  - HTF vs. G5
  - First results available in a few months
- Medium study 2
  - G5 vs. Quinn's advantage
  - Currently set up
  - You all will be invited soon
- Effect of culture conditions on embryo transcriptome (AMC/MUCM/UMCG)
  - Culture media
  - Oxygen concentration



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Hesham Al-Inany

Choosing an IVF culture medium
Check before you choose
Randomize while you use

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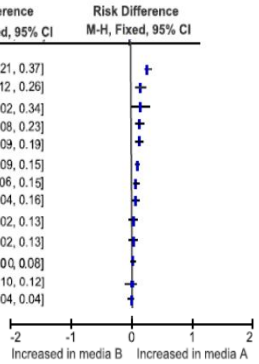
Good quality embryos

Study	Media A vs. Media B		Media A Events	Media A Total	Media A %	Media B Events	Media B Total	Media B %	Risk Difference M-H, Fixed, 95% CI
Number of good quality embryos									
Di Falco Cossiello et al. 2011	Global	Universal IVF	80	199	(40.2)	29	260	(11.2)	0.29 [0.21, 0.37]
Di Falco Cossiello et al. 2011	Global	HTF	80	199	(40.2)	249	1170	(21.3)	0.19 [0.12, 0.26]
Hoogendijk et al. 2007*	Quinn's Adv	Sydney IVF	40	67	(58)	33	79	(41.8)	0.18 [0.02, 0.34]
Di Falco Cossiello et al. 2011	Global	IVF	80	199	(40.2)	165	660	(25)	0.15 [0.08, 0.23]
Di Falco Cossiello et al. 2011	IVF	Universal IVF	165	660	(25)	29	260	(11.2)	0.14 [0.09, 0.19]
Balaban et al. 2005*	G3	G2	1070	1694	(63.2)	895	1754	(51)	0.12 [0.09, 0.15]
Di Falco Cossiello et al. 2011	HTF	Universal IVF	249	1170	(21.3)	29	260	(11.2)	0.10 [0.06, 0.15]
Paternot et al. 2010*	Sydney IVF	GM501	169	419	(40.3)	177	583	(30.4)	0.10 [0.04, 0.16]
Hambiliki et al. 2010	G5	EmbryoAssist	233	382	(61)	174	315	(55.2)	0.06 [-0.02, 0.13]
Artini et al. 2004	P1	HTF	229	320	(71.6)	194	294	(66)	0.06 [-0.02, 0.13]
Di Falco Cossiello et al. 2011	IVF	HTF	165	660	(25)	249	1170	(21.3)	0.04 [-0.00, 0.08]
Parinaud et al. 1999	SMART2	EllioStep2	71	143	(49.7)	72	148	(48.6)	0.01 [-0.10, 0.12]
Ben-Josef et al. 2004*	P1	Sydney IVF	630	746	(84.4)	523	621	(84.2)	0.00 [-0.04, 0.04]



Fertilization rate

Study	Media A vs. Media B		Media A Events	Media A Total	Media A %	Media B Events	Media B Total	Media B %	Risk Difference M-H, Fixed, 95% CI
Fertilization rate									
Paternot et al. 2010*	GM501	Sydney IVF	583	788	(74)	419	669	(62.6)	0.11 [0.07, 0.16]
Zollner et al. 2004*	G2	BlastAssist	914	1044	(87.6)	712	890	(80)	0.08 [0.04, 0.11]
Artini et al. 2004	P1	HTF	320	512	(62.5)	294	502	(58.6)	0.04 [-0.02, 0.10]
Campo et al. 2010*	GM501	ISM1	433	595	(72.8)	446	652	(68.4)	0.04 [-0.01, 0.09]
Parinaud et al. 1999	SMART2	EllioStep2	143	236	(60.6)	148	257	(57.6)	0.03 [-0.06, 0.12]
Khoury et al. 2012-	Global	Quinns's ad	504	568	(88.7)	513	594	(86.4)	0.02 [-0.01, 0.06]
Staessen et al. 1998	MB2	Universal IVF	1495	2377	(62.9)	1485	2435	(61)	0.02 [-0.01, 0.05]
Balaban et al. 2005*	G3	G2	1727	2361	(73.1)	1785	2499	(71.4)	0.02 [-0.01, 0.04]
Mauri et al. 2001*	P1	IVF	725	965	(75.1)	751	1010	(74.4)	0.01 [-0.03, 0.05]
Findikli et al. 2004	ISM	G2	524	717	(73.1)	520	717	(72.5)	0.01 [-0.04, 0.05]
Utsunomiya et al. 2002*	HTF	G2	536	936	(57.3)	178	312	(57.1)	0.00 [-0.06, 0.07]